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THE SPORE-MOTHER-CELL OF ANTHOCEROS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XV.

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(WITH PLATES IX AND X)

ANYONE who has ever examined sections of the sporogonium of Anthoceros must have been impressed with the extreme beauty of the spore-mother-cells as they are exhibited in all stages of development throughout the length of this structure. The exceptional chloroplast and the various conditions illustrating the division of the cell-contents to form the spores are perhaps the most striking features presented. These led the writer to attempt a detailed examination in the hope that light might be thrown on certain problems that interest the student of plant cytology

The work of Farmer ('94 and '95) is the only contribution of a detailed nature upon the cytology of the Hepaticæ. He did not study Anthoceros but confined himself chiefly to certain thalloid Jungermanniaceæ and reported some very interesting and remarkable conditions. The present investigation does not agree with his accounts of the processes of nuclear division presented by such forms as Pallavacinia, Aneura, Fossombronia, and Pellia. However, at the outset the writer wishes to express no surprise at important differences between such divergent forms as Anthoceros and members of the Jungermanniaceæ. The researches of the past few years in cytology have certainly taught us to be wary of generalizing upon the conditions illustrated by particular cases.

This investigation deals with the single species *Anthoceros laevis* L., the material being collected at Woods Hole, Mass., where were conducted the preliminary studies and experiments on methods of fixation. The best results were obtained from specimens that had been fixed for twenty-four hours in a weak Flemming solution of the following formula: 1 per cent. chromic acid 25^{cc}, 1 per cent. glacial acetic acid 10^{cc}, 1 per cent. osmic acid 10^{cc}, water 55^{cc}. Much material, however, was killed in other ways, sectioned and stained to determine as precisely as possible the merits of Flemming and the disadvantages of other killing agents. The results of these experiments are brought together at the end of the paper and present some interesting data.

Sections were cut 5 and 7.5 μ thick from material embedded in paraffin and for the most part stained after Flemming's method with safranin, gentian violet and orange G. Excellent results were obtained with iron alum and haematoxylin employed according to the principles of Haidenhain. Acid fuchsin followed by methyl green or gentian violet presented interesting examples of differential staining, but the results were not as good as those given by Flemming's triple stain.

At the outset it will be well to state that the number of chromosomes for *A. laevis* appears to be four and eight in the gametophyte and sporophyte respectively. The count in the gametophyte is based solely upon observations of many mitotic figures in the cells of a developing antheridium, but it agreed with the number presented in the nuclear figures in the spore-mother-cell. The number eight for the sporophytic generation was presented quite frequently and in different regions of that structure. Thus the archesporium just before the differentiation of the spore-mother-cells, the tissue below the archesporium, and the cells of the columella afforded many nuclear figures in which a count could be made with reasonable accuracy.

The attractiveness of the sporogonium of Anthoceros as a subject for study lies largely in the fact that in one structure there may be present a very complete series of stages from the time when the spore-mother-cell is differentiated until the spores are fully ripe. It is possible from this fact to relate to each other with great exactness the events that take place as the spore-mother-cell matures.

As is well known, the sporogonium of Anthoceros grows from a region of embryonic or meristematic tissue in the slightly constricted portion of the structure just above the foot. From this region there rises a central columella around which are arranged in the form of a hollow cylinder the cells destined to develop into spores and sterile cells (elaters). Recently published accounts of the structure and development of the sporogonium by Campbell (**'95**, pp. 130–131) describe this hollow cylinder as two layers of cells thick and composed of groups of spore-mother-cells separated by clusters of sterile cells that later become the elaters. The writer's preparations indicate far less regularity of structure than this description would imply. In many cases the spore-mother-cells formed a single layer around the columella and the amount of sterile tissue was very insignificant. Only occasionally was the layer of cells distinctly double and then very irregularly so. There appears, therefore, to be considerable variation in the complexity of the sporogenous tissue in Anthoceros, and the simplest conditions approach very closely the arrangement of cells reported by Campbell (**'98**) for Dendroceros.

The cubical cells of the archesporium frequently contain nuclear figures, but the spindles are very small and unsatisfactory for study. Such a spindle is shown in *fig. 1*, with the chromosomes separating into two groups and about to pass to the poles. The cytoplasm is granular and contains minute vacuoles. The writer was not able to find a trace of the chloroplast in these cells, but looks forward to a further study of this point in living material and with special methods of staining.

The spore-mother-cells become very quickly differentiated by a rapid increase in size. Their nuclei are not at first to be distinguished from those in the cells of the archesporium, for they are small and contain the one prominent nucleolus and an ill-defined network of linin threads. The most conspicuous change in the cell is the appearance in fixed material of the chloroplast. This structure is at first a slightly denser mass of protoplasm than the other contents of the cell, but so small that it can scarcely be distinguished from differentiated granular regions of the cytoplasm. Indeed, the first clear proof of its presence in my preparations has been the sharp staining of the starch grains (purple with gentian violet) that are found almost immediately in the interior. The reader may see in *fig. 3* such an early stage in the development of the chloroplast, and should notice that the three small starch grains lie in a homogenous matrix of much the same consistency as the surrounding cytoplasm. The chloroplast in cells younger than that shown in *fig. 3* could not be recognized with certainty by the methods of staining employed in the present study.

The chloroplast once differentiated soon becomes the most conspicuous object in the spore-mother-cell by virtue of its size and brilliant differentiation with stains (*figs. 4, 5*). The peculiarities become more apparent as it enlarges. The outline is sharply marked but it has been impossible to establish a distinct bounding membrane. The interior contains beautifully defined starch grains that become very numerous as the chloroplast increases in size and finally fill by far the greater part of the enclosed region. Each starch grain at first occupies a little cavity surrounded by delicate films of protoplasm that appear as strands in the sections figured (*figs. 5–9*). The chloroplast has therefore a honeycomb-like structure, each cavity being entirely or almost entirely filled by a grain of starch.

The structure of the protoplasmic strands and films around the starch grains proved a very difficult subject for study. They are granular and extend on all sides to the boundary of the chloroplast where they merge into the densely staining film at the exterior. But the bounding film grades off insensibly into the cytoplasm and it was not possible to distinguish by any stain reaction the strands inside the chloroplast from the protoplasm outside.

The first division of the chloroplast takes place very quickly after the structure reaches a certain size. Preparation for the process is usually indicated by the chloroplast elongating and bending around the nucleus in the form of a thick crescent.

The phenomenon of division is one of simple constriction in the middle region and final separation of the halves as the furrow deepens around the chloroplast. It is very plain that the line of fission is determined by the furrow and not from the interior of the chloroplast. Therefore if any particular region of the protoplasm is actively concerned in this process one would naturally first seek the agent in the peculiar film bounding the chloroplast. However there is little in the structure of this region to indicate such important activities.

After the first division of the chloroplast the two portions usually separate and pass to opposite ends of the elongated spore-mother-cell (*fig. 6*). Each has a structure quite similar to the parent chloroplast except that the starch grains become even more numerous and prominent and the fine strands and films of protoplasm separating the starch grains are less clearly defined. Soon after this first division of the chloroplast there takes place that peculiar condition of the nucleus termed synapsis, but this phenomenon had best be considered in a later portion of the paper that deals with nuclear activities. There is a well defined period when the spore-mother-cell has only two chloroplasts, and no longitudinal section of the sporogonium will fail to exhibit a region in which all of the cells are in this condition. As a spore-mother-cell grows older, and increases still further in size, indications of the approaching second division of the chloroplast become manifest.

The second division of the chloroplast repeats exactly the phenomenon of the first division. As a rule both structures concerned are active at the same time, but one may be in a more advanced state of fission than the other. The proportions of the spore-mother-cell at this time are more nearly equal (*fig. 7*) than in the previous condition, *i. e.*, the cell is broader in proportion to its length. As a result the four chloroplasts are distributed around the centrally placed nucleus in such a manner that each occupies the greater part of one of the four tetrahedral divisions of the spore-mother-cell that are present after the separation of the cell contents to form the spores. Sometimes the second division of the chloroplast follows so closely after the first that the final products are arranged for a short time in the form of a bow around the nucleus (*fig. 9*). Such conditions are however only transitory and result from the elongated forms of such cells.

The structure of the four daughter chloroplasts exhibits this difference from that of the mother structure shown in *figs. 4, 5*. The protoplasmic material that formerly filled the spaces between the starch grains as a spongy network of strands and films now appears to be entirely absent or present only in a much reduced form. The chloroplast has the structure of a vesicle filled with starch grains. It is interesting to contrast this condition with the conditions exhibited in the youngest spore-mother-cells. There the bulk of the chloroplast was made of protoplasm and the few starch grains were insignificant. But in the oldest spore-mother-cells the chloroplast has become one great storage vesicle of starch, conspicuous by the absence of that protoplasmic differentiation which is so characteristic of the chloroplast wherever found.

The problem of the division of the chloroplast and the distribution of the resultant daughter plastids through the cell is full of interest. Does the development of the furrow and the final fission of the chloroplast result from the activity of the protoplasm in or around the structure? It is conceivable that the division has its cause merely in the advantage that might come from a more even distribution of the chlorophyll through the cytoplasm. Thus it is obvious that the arrangement shown in *fig. 5* is very unsymmetrical. Perhaps a strain may be exerted upon a chloroplast bent around the nucleus in such a manner and finally result in its fission, and then lead to a redistribution of the cell contents to bring about a certain balance in the cell. The difficulty of this view as applied to Anthoceros lies in the fact that the number of chloroplasts is fixed at four, corresponding exactly to the number of spores. How could a numerical coincidence so important in phylogeny be left to the play of forces merely acting for symmetry and the advantageous distribution of chlorophyll throughout the cell? But if we assign the cause of the division to the protoplasm in or around the chloroplast there arise complications very difficult to explain. The four chloroplasts are in the cell long before the nucleus divides. Can it be supposed that cytoplasm would be intrusted with so important a task as the preparation of a chloroplast for each of the four nuclei that are later to preside over the spores before there is any indication that such nuclear division is to take place? The process reverses what would appear to be the natural course of events; *i. e.*, one would suppose that the division of the nucleus would determine the position of the four spores and that the cell contents would arrange themselves later with reference to the nuclei. Perhaps this really is the fact, and it may be merely chance that each of the four nuclei finds a single chloroplast to accompany it in the spore, but such coincidence of numbers would be very curious. A somewhat similar problem is presented by the oospore of Coleochaete, where, according to Oltmanns, eight chloroplasts are formed before the nucleus divides, and then as cell division progresses the nuclei are distributed symmetrically until each becomes associated with one chloroplast.

The structure and behavior of the chloroplast suggests some extremely interesting lines of research. Is there such a substance as plastidplasm, a particular form of protoplasm with morphological characters that may distinguish it from trophoplasm, filarplasm, centrospheres, and other differentiated structures in the cell? If there is a plastidplasm, what form does it assume at the periods of ontogeny, when chlorophyll and other pigments are absent? Is the plastid a permanent organ of the cell, as is perhaps generally supposed? As the plastid is a region of the protoplasm where the pigment is gathered, and consequently the seat of metabolic activity, it is possible that these functions might produce its form and structure. It is even conceivable that the differentiation of a plastid may not lie in peculiarities of protoplasm itself, but represent the outward effects of the metabolic phenomena concerned with its pigment.

It appears to the writer that plant cytology has a very important field open for investigation dealing with the structure of the chromatophore and its place in ontogeny.

We pass now to the second part of the paper, which is to consider the behavior of the nucleus in sporogenesis. The structure of the spore-mother-cell and process of nuclear division in Anthoceros was first described by Strasburger (**'80**, p. 162). The following account entirely supports the essentials of those observations, but the present investigation attempts a more detailed examination of nuclear activities, involving the problems of spindle formation, synapsis, the succession of nuclear divisions, and formation of the walls between the spores.

The fact has already been stated that eight chromosomes are present in the sporophyte, and consequently enter the nucleus of the spore-mother-cell. This nucleus in a resting condition is very similar to the nuclei of the archesporium and shows very little structural differentiation (*fig. 3*). It is small and its linin network is very inconspicuous, but the nucleolus is prominent.

Coincident with the appearance and increase in size of the chloroplast the nucleus enlarges (*figs. 4, 5, 6*) and the threads of linin become very prominent. They are so exceedingly small, however, that it was not possible to determine accurately the structure, even with a Zeiss apochromatic immersion lens under the magnification of 2250 diameters. Dark-staining minute bodies along the linin thread are presumably granules of chromatin, but their distribution could not be ascertained with certainty, nor was it possible to follow the convolutions of the spirem.

The condition of the nucleus after the first division of the chloroplast is shown in *fig. 6*. The spirem thread is strongly developed, but the spotted character of the nucleolus indicates that the latter structure may be the seat of internal changes. Suddenly, as indicated by the rarity of intermediate conditions, the nucleus passes into the state of synapsis. The spirem thread contracts into an irregularly shaped mass that usually lies by the side of the nucleolus. This condition is illustrated in *fig. 7*. Synapsis in Anthoceros seems to occur after the first division of the chloroplast, and it is very apt to persist through to the second division. The contracted threads of linin then gradually extend outward through the nucleus (*fig. 8*), and finally a spirem condition is attained similar in all outward appearances to that present in the nucleus before synapsis.

The nucleolus after synapsis is often found somewhat fragmented, as is shown in *fig. 10*. Whether or not the nucleolus partly dissolves and contributes material to the linin thread, as has been suggested for certain forms (*e. g.*, Lilium), could not be determined for Anthoceros.

Synapsis in Anthoceros does not appear to the writer to be an artifact, and in this opinion he agrees with the views expressed by a number of investigators who have studied this phenomenon in other types. Particularly favorable opportunities are presented for the solution of this problem in Anthoceros, because one may subject at one time all conditions of the spore-mother-cells to the same fixing fluid. Under these conditions synapsis always appears in that particular region of the sporogonium comprising cells in the condition just before and during the second division of the chloroplast. No nuclei were ever observed in synapsis in other parts of the sporogonium, and nuclei in younger and older cells immediately adjoining the disturbed region presented the typical fully expanded spirem thread. Yet all of these cells had been bathed in the same fixing fluid and experienced identical treatment in the preparation of the slides. Of course it may be claimed that the contents of the nuclei are more subject to shrinkage at the time when synapsis appears, but it should be understood that such a variety of fixing fluids as Flemming's, picro-acetic, corrosive sublimate, chrom-acetic, and Merkel's, all gave identical results. Unfortunately the small size of the nuclear elements in Anthoceros made a detailed study of this interesting process extremely difficult, and led to no definite conclusions as to the meaning of synapsis.

We have seen that events take place during the growth of the spore-mother-cell in the following order: *first*, an increase in size of the nucleus, the assumption of the spirem condition, and the first division of the chloroplast; *second*, sudden synapsis at the time when there are two chloroplasts in the cell, and often continuing until after the second division of these structures; *third*, gradual emergence of the nucleus from synapsis. These changes present the spore-mother-cell ready for nuclear division with the following arrangement of the cell contents. The four chloroplasts are distributed symmetrically, with the nucleus in the center of the cell. In this condition the cell remains for a period somewhat longer than that of the synapsis, and then prepares for the first division of the nucleus.

It is difficult to recognize the earliest indications that the nucleus is approaching prophase of division. A somewhat later stage is very conspicuous, when the outline of the nucleus becomes angular and a mat of delicate threads surrounds the structure (*fig. 12*). There is a period, however, previous to this condition, when the nuclear membrane appears much less firm and somewhat irregular in outline. This structure follows the spirem stage such as is shown in *fig. 10*, and precedes the unmistakable prophase conditions illustrated by *figs. 12, 13*. Its appearance is given in *fig. 11*, and the following peculiarities should be noted, viz., an irregularly outlined nuclear membrane, a faint linin network, and fragmentary nucleolus. But perhaps the most important characteristics appear outside of the nuclear membrane in the cytoplasm as a delicate web of fibrils closely applied to the nucleus. These fibers are so delicate as almost to defy the reproduction that has been attempted in *fig. 11*. They presage the development of the spindle.

In the prophases the nucleus may exhibit some extremely varied appearances. The form is very irregular, taking on curious wavy angles. Outside of the nucleus there is a region of protoplasm manifestly very different in structure from the neighboring cytoplasm. In overstained preparations this region is very conspicuous, forming a sort of zone around the nucleus, but the details of its structure can only be made out in very favorable preparations. Then it is seen to possess a fibrillar structure. The delicate threads lie all around the nucleus, but they accumulate conspicuously at the pointed projections (*figs. 12, 13*).

At this time one is apt to find the linin network contracted away from the nuclear membrane and gathered in the form of a confused tangle in the central region somewhere near the nucleolus (*fig. 13*). The chromatic material becomes more prominent and from the ill-defined mass in the interior there emerge four deeply staining chromosomes (*figs. 12, 14*). When the chromosomes are fully differentiated one may expect to find one or two of the angles of the nucleus more prominent than the others, and the fibrils around them taking on the appearance of spindle fibers (*figs. 13, 14*). The process of spindle differentiation is a gradual one, and only in the later stages is it possible to feel suré of the direction that the axis will assume. It is seldom that the two poles appear from the beginning so nearly opposite one another as to have a common axis. It is more usual for the spindle to be somewhat bent at first, as is shown in *fig. 14*. However, ultimately the two poles arrange themselves to form a symmetrical spindle of the form illustrated by *figs. 15, 16, 17, 18*.

The fully developed spindle has an interesting structure, with several clearly marked features well shown by *fig. 18*. The poles are flat, or perhaps slightly convex; they are never pointed. Mantle fibers are conspicuous around the nuclear plate (*figs. 16–18*), but what relation these bear to the chromosomes could not be determined.

In view of the condition reported by Farmer ('94 and '95) for a number of the Hepaticae, notably Pellia, Pallavicinia, and Fossombronia, it became a very important problem to determine whether or not centrospheres were ever present at the poles of the spindles. The writer searched his preparations with great care for these structures, but came to the conclusion that they do not exist in Anthoceros. Sometimes the cytoplasm around the spindle contains large deeply staining granules, such as are shown in *fig. 17*, and these may occupy positions near the ends of the spindles, but extensive observations would convince anyone that they have nothing whatever to do with centrosomes. The protoplasm in the vicinity of the pole is often very dense in structure, containing no granules and few vacuoles. It frequently presents the appearance shown in *fig. 16*, but it never exhibited indications of that differentiation expected of a centrosphere.

It will be apparent to the reader from this account that the spindle of Anthoceros during the first division follows a history closely parallel to that described for homologous cells in Lilium by Mottier ('97), Hemerocallis by Juel ('97), Equisetum by Osterhout ('97), and Cobæa by Lawson ('98). That is to say, the spindle is organized by numerous delicate fibrils of protoplasm that develop conspicuously during prophase in the cytoplasm around the nuclear membrane. The fibrils are at first somewhat irregularly distributed, but finally become arranged in the form characteristic of the respective spindles.

Strasburger in the third edition of the *Lehrbuch der Botanik*, 1898, p. 67, has introduced the term filarplasm to be applied to protoplasm having the form and activities above described and so clearly established by the researches of his students during the past three years. The writer understands that filarplasm is supposed to be made up of the substance designated by the older term kinoplasm. However, filarplasm has morphological characters, as indicated by its name, and these are the thread or fibril-like structures. The term therefore expresses admirably the facts of morphology without implying or assigning physiological activities to the substance.

It will be very gratifying if future investigation should establish filarplasm as an element in the cells of higher plants differentiated from other forms of protoplasm. This investigation appears to extend the range of mitoses associated with filarplasm into a group of plants much lower than the lowest previously reported (pteridophytes by Osterhout). It is also significant that it should be a class containing one large order, the Jungermanniaceæ, where according to Farmer centrospheres are very marked features of the mitotic figures. There should be types of plants that will allow us to form some idea of the relationship between filarplasm and the asters and centrospheres present in certain thallophytes, in other words solve the problems of their places in phylogeny. Perhaps the future study of Hepaticæ will throw some light on this question.

The two sets of daughter chromosomes that result from the division at metaphase pass very quickly from the nuclear plate to the poles of the spindle. Here they may frequently be found clustered together in a region of dense protoplasm that surrounds the group, as is shown in *fig. 19*. The development of a nuclear membrane around the chromosomes finally organizes the two daughter nuclei which are connected for a short time by the spindle fibers. These latter structures gradually fade away, beginning at the poles (*fig. 20*), and finally entirely disappear. The daughter nuclei then lie in undifferentiated cytoplasm usually occupying positions between the chloroplasts somewhat as is indicated in *fig. 21*.

The daughter nuclei following the first division pass into a fully developed resting condition. The chromosomes break up into a number of chromatin granules that become distributed over a linin network. A prominent nucleolus appears. In sections of a sporogonium one may always find spore-mother-cells containing two nuclei, although this condition is quickly replaced by the succeeding second division.

The second mitosis in the spore-mother-cell involves both nuclei simultaneously, but the two nuclear figures are developed independently of one another. The two spindles are never united by cross fibers. Sometimes the two spindles will lie almost side by side, but usually they are placed at right angles to each other. Frequently one section of a spore-mother-cell will give a polar view of one spindle and the neighboring section a longitudinal view of the other. An instance of this character has been chosen to illustrate the principal features of their structure and is shown in *fig. 24*. By comparing *fig. 24* with illustrations of the first division (*figs. 16–18*) it will be seen that the spindles of the second mitosis are much smaller. However they exhibit an essentially similar structure, having flattened poles without centrospheres. Several prominent fibrils make up the center of the spindle which is bordered by an ill-defined set of mantle fibers, The polar view of a spindle shown at the left of *fig. 24* demonstrates that four chromosomes are present at the nuclear plate, the same number that appears during the first mitosis. Prophase and anaphase conditions of the second mitosis are found only with great difficulty and are very unsatisfactory for study because of the small size of the elements involved.

The four nuclei that result from the second mitosis associate themselves each with one chloroplast, and with these become distributed symmetrically through the cell, so that the protoplasm naturally segregates into four regions representing what are later to become the tetrahedral division of the spore-mother-cell (*figs. 26, 27*). The spindle fibers disappear completely.

The problem of the splitting of the chromosomes engaged the writer's attention, but it must be plain that Anthoceros is not a favorable subject for the study of this process. The nearly spherical form of the chromosomes offers immense difficulties in orientation. Farmer ('95) reported some peculiar conditions in the forms studied by him, which he considers as illustrations of the "heterotype" division described by Flemming. They result from the habit that the chromosomes have of doubling on themselves and then being pulled apart as V-shaped daughter chromosomes. The figures are very complex, but Farmer assures us that the division is really longitudinal and not transverse, so that it cannot be interpreted as qualitative. In Anthoceros all evidence that the writer can present indicates that the splitting in both mitoses is longitudinal and after the normal type. Polar views of spindles after the splitting of the nuclear plate sometimes present the daughter chromosomes, eight in number, arranged in four pairs. Such a stage is shown in *fig. 23*, and the grouping certainly indicates that each of the original four chromosomes has divided longitudinally into halves. It is not at all unusual to find the two sets of daughter chromosomes placed as in *fig. 22*, which might suggest transverse fission, but the writer is not willing to accept this evidence, as such an arrangement would naturally appear when the daughter chromosomes pass away from the nuclear plate to the poles of the spindle.

We have now finished our account of nuclear activities in the spore-mother-cell, but there remains for consideration the description of the manner in which the cell contents are divided to form the spores. After the two mitoses each of the four nuclei lies in a region of dense protoplasm at the side of a chloroplast towards the interior of the cell (*fig. 25*). The bulk of the protoplasm is therefore collected into four masses somewhat apart from one another but connected by very numerous delicate filaments. The cross filaments are very conspicuous but irregular in their arrangement, frequently anastomosing. As shown in *fig. 25* they are not confined to the vicinity of the nuclei but connect all portions of the separated regions of protoplasm. They do not resemble spindle fibers, being much thicker, but have instead the appearance of strands of cytoplasm. Following the condition shown in *fig. 25* one may find stages similar to *fig. 26*. It is plain that the protoplasmic strands have spread sideways and fused with one another so that there is now present a film of protoplasm between tetrahedral regions of the spore-mother-cell. This film marks exactly the position that is finally to be occupied by cell walls when the spores are fully organized.

The peculiarity of the process just described lies chiefly in what seems to be its entire independence of spindle fibers. It is the generally accepted view that the walls crossing spore-mother-cells and pollen-mother-cells are derived from cell plates that result from the fusion of spindle fibers after anaphase. The writer thinks that no investigator has described a condition similar to Anthoceros. The present studies indicate that the spindles of the two successive mitoses completely disappear. This is certainly a very difficult point to determine, but the writer feels confident that the anastomosing strands which connect the four masses of protoplasm in the spore-mother-cell, as has been shown in *figs. 25, 26*, are formed entirely independently of spindle fibers. How then are the walls formed?

It is certain that the film of protoplasm indicated in *fig. 26* thickens and finally gives place to a straight wall, at first delicate (*fig. 27*) but gradually becoming firmer until its cellulose nature is unmistakable.

The phenomenon is typical of one of the processes recently discussed by Strasburger ('98), in which a cell wall is formed in the interior of the protoplasm. It appears as if the film of protoplasm exhibits the activities present in the "Hautschicht" when it lays down or increases the thickness of a cell wall in the manner known as apposition, involving, at least in part, the change of its own substance into cellulose.

With the separation of its contents the spore-mother-cell as a unit ceases to exist, and a new set of activities begins that may very properly be reserved for discussion in another paper. Some interesting events take place in the spore as it ripens, but technical difficulties interfere greatly with their elucidation.

The peculiar fact that the sporogonium of Anthoceros presents spore-mother-cells in all stages of development makes it possible to contrast the times occupied by the various changes. One cannot establish the actual duration of any process, but within certain limits it is possible to determine the relative periods of each event. It must be assumed that the rate of growth during the season is approximately uniform. The unit of the calculation must be the time necessary to differentiate one spore-mother-cell from the archesporium. As the result of a laborious examination and count the writer ventures to present the following time schedule of the events which take place in the spore-mother-cell. From 12–18 units of time are necessary to bring the spore-mother-cell to the period of the first division of the chloroplast. The division of the chloroplast occupies 6–10 units. The nucleus is in synapsis 12–20 units. For 30–50 units the cell has two chloroplasts. It takes about 25 units after synapsis to produce conditions favorable for mitosis. The first mitosis occupies 1–3 units, and the two daughter nuclei rest for 2–4 units before the second mitosis which is more rapid than the first. There is finally a period of 3–6 units before the spores are definitely organized by the partition walls across the spore-mother-cell.

The results presented by this investigation indicate that the Hepaticæ are likely to furnish some very interesting material for future researches in cytology. From superficial examination it appears probable that the Ricciaceæ and Marchantiaceæ in general present mitoses similar to Anthoceros, but we may hope that some forms exist that will harmonize the peculiarities described for the Jungermanniaceæ with the conditions found in Anthoceros.

EXPERIMENTAL TECHNIQUE.

As stated in the beginning of the paper the writer experimented with a number of fixing agents to determine as precisely as possible their merits or faults. Of these Flemming's formula designated "weak" gave decidedly better results than any other. In Anthoceros, and presumably for other types, the chief test of a fixing fluid is its effect upon the achromatic parts of a nuclear figure. Chromosomes are the least difficult of all the nuclear elements to preserve. Preparations will not infrequently present beautiful views of nuclear plates when the spindles are manifestly in very bad condition. The following is a brief statement of the effects of several fluids upon nuclear figures in the spore-mother-cell.

Chrom-acetic acid fixes filarplasm but the safranin stains diffusely after it, and gentian violet does not hold well in the spindle fibers. If sections fastened to the slide be left several days in weak Flemming the staining qualities with safranin and gentian violet are much improved although it is doubtful if they can be made as good as those presented by Flemming fixed material.

Merkel's fluid (1 per cent. chromic acid 12^{cc}, 1 per cent. platinum chlorid 12^{cc}, water 72^{cc}) even when used for long periods (36 hours) is thoroughly unsatisfactory. The spindles are badly fixed.

Boveri's picro-acetic acid gives beautifully bleached tissue, but achromatic regions are not clearly differentiated although chromatic elements stain well.

Sublimate-acetic (5 per cent. glacial acetic acid in saturated solution of corrosive sublimate) is not good. Nuclear membranes and filarplasm are very poorly preserved.

Hermann's fluid is very much like Flemming's in its effects and is thoroughly satisfactory.

The osmic acid of the Flemming's and Hermann's mixtures appears to give them certain advantages over all other fluids. Although they may not kill and preserve tissue better than some other agents, as for example chrom-acetic acid, certain stains, safranin and gentian violet, differentiate all structures of the cell very much better when they have been used.

SUMMARY.

The number of chromsomes is eight for the sporophyte and four in the gametophyte.

The chloroplast appears rather suddenly in the spore-mother-cell as a differentiated region of the protoplasm, containing several starch grains. When fully developed it has a honeycomb structure, each cavity being occupied by a grain of starch.

The division of the chloroplast is one of simple fission, apparently through forces acting outside of the structure, and perhaps concerned with the film of protoplasm that surrounds it.

Synapsis occurs in the nucleus soon after the first division of the chloroplast. It is not an artifact.

The second division of the chloroplast presents the spore-mother-cell ready for the division of the nucleus. The four chloroplasts, hardly more than vesicles filled with starch grains, are arranged symmetrically in the cytoplasm with the nucleus in the center of the cell.

The resting nucleus has a nucleolus and a spirem thread, which, however, is so small that details of structure could not be determined.

Prophase conditions of the nucleus show the presence of a mesh of delicate fibrils (filarplasm) around the structure. The nuclear membrane is at first irregularly angular, but finally two poles of a spindle are differentiated.

The metaphase presents a spindle with flattened poles, entirely lacking bodies that might be interpreted as centrospheres or centrosomes.

There is a period of rest after the first mitosis when each daughter nucleus has a nucleolus and spirem thread. The two mitoses are, therefore, successive.

The second mitosis presents structural features quite identical with the first.

The chromosomes are four in number in each mitosis. They appear to split longitudinally.

All traces of the spindles become lost soon after each mitosis.

The fully mature spore-mother-cell presents four chloroplasts, each with a single nucleus on the interior side. The protoplasm segregates in these four regions of the cell, leaving spaces that are crossed by many anastomosing strands of cytoplasm. These strands cannot be traced from spindle fibers and appear to have no connection with filarplasm.

The walls separating the cell contents into four spores are derived from films of protoplasm that appear between the chloroplasts with their respective nuclei. The films are formed by the coalescence of strands of cytoplasm that cross the spaces between the four regions of cell contents.

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EXPLANATION OF PLATES IX AND X.

All preparations were studied under a Zeiss oil immersion 2^{mm} aper. 1.30 with compensation-oculars. Figures were sketched with an Abbé camera at the following magnifications, *figs. 3–7, 9, 10, 12, 16–21, 24–27*, magnified 1000 diameters; *1, 2, 8, 11, 13, 14, 15. 22*, 1500 diameters and *fig. 23*, 2250 diameters.

All preparations except that shown in *fig. 2* from material fixed in weak Flemming, sectioned 5μ thick and stained on the slide; *figs. 1, 15, 18, 22* and *23* from slides stained with iron-alum-haematoxylin after the method of Haidenhain; all other figures from preparations stained with Flemming's triple stain, safranin, gentian violet, and orange *G*.

PLATE IX.

FIG. 1. Nuclear figures from archesporium, just after the splitting of the chromosomes whose number is large, apparently about sixteen, eight for each daughter nucleus.

FIG. 2. Nuclear figure from antheridium, four chromosomes.

FIG. 3. Spore-mother-cell, early condition of chloroplast with three or four grains of starch.

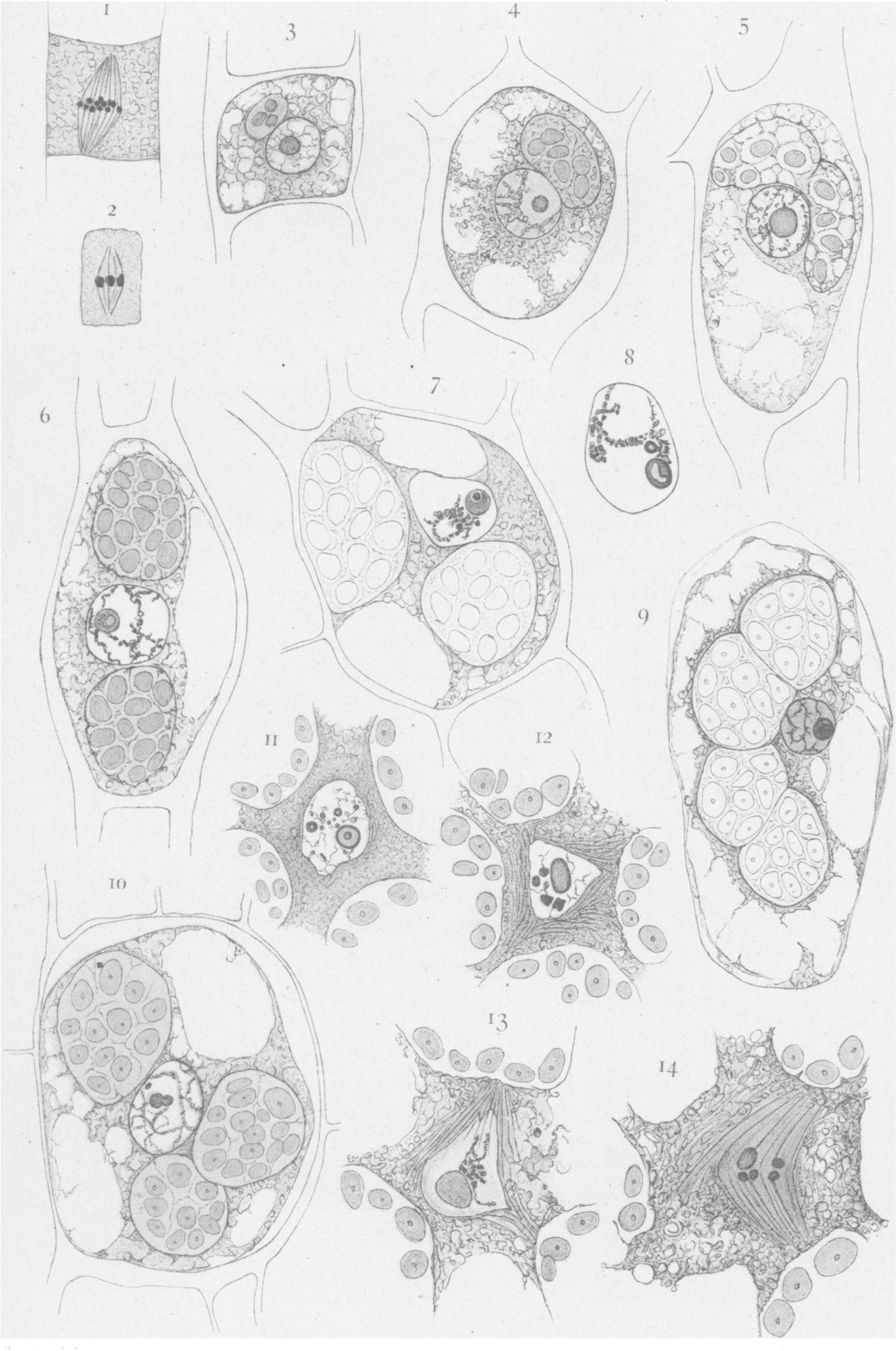
FIG. 4. Spore-mother-cell in more advanced condition than *fig. 3*, linin network more prominent, chloroplast elongating preparatory to division; starch grains prominent.

FIG. 5. First division of chloroplast.

FIG. 6. Two chloroplasts, linin network prominent.

FIG. 7. Synapsis, two chloroplasts.

FIG. 8. Nucleus emerging from synapsis.



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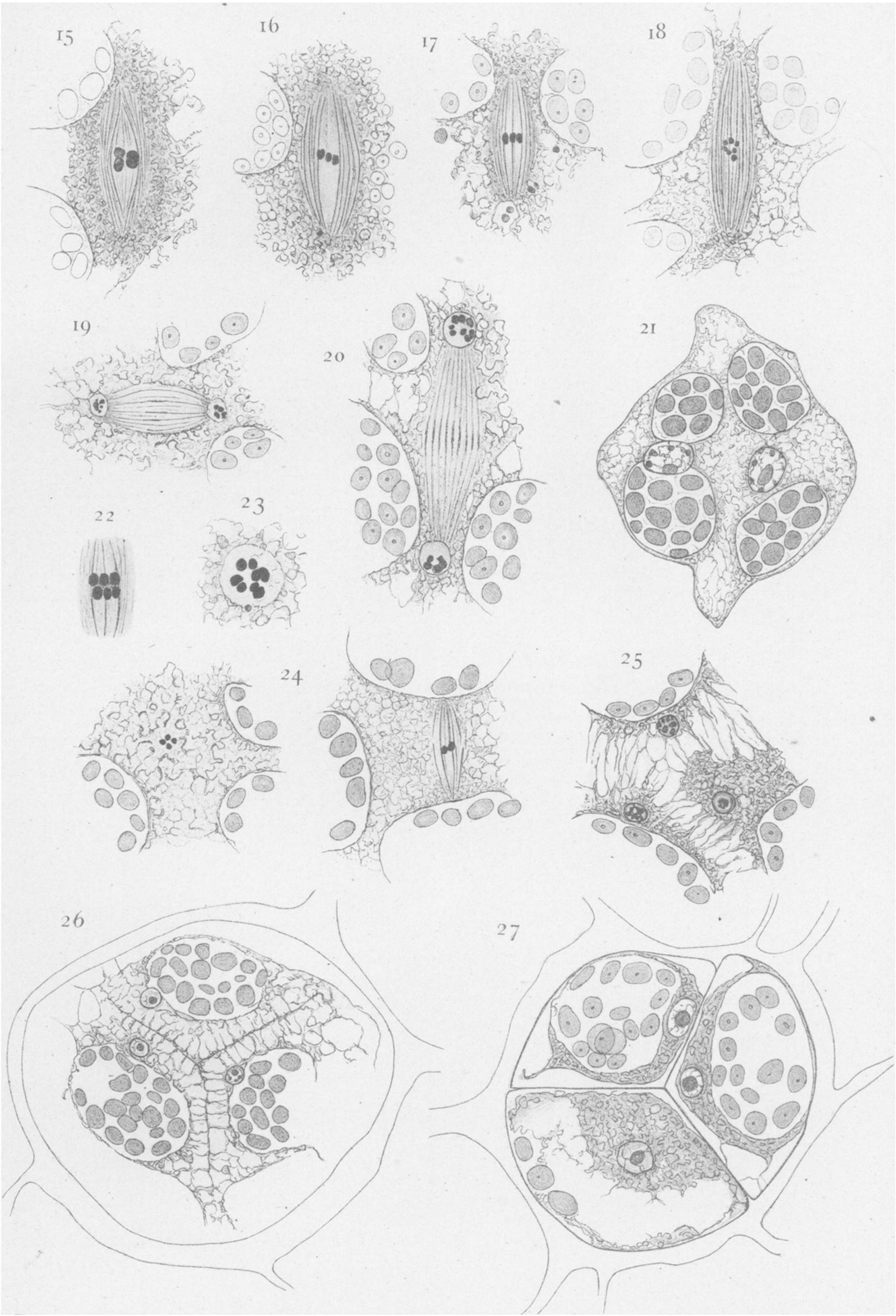

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FIG. 9. Second division of the chloroplasts.

FIG. 10. Spore-mother-cell at maturity, four chloroplasts.

FIG. 11. First indication of approaching mitosis, accumulations of filarplasm around the nucleus whose membrane appears less clearly defined.

FIG. 12. Prophase, filarplasm well differentiated.

FIG. 13. Prophase, one pole of spindle developed.

FIG. 14. Late prophase, the two poles of the spindle differentiated, four chromosomes assembled in the equatorial region.

PLATE X.

FIG. 15. Early metaphase, four chromosomes at nuclear plate.

FIG. 16. Metaphase, strongly developed mantle fibers.

FIG. 17. Metaphase, granular cytoplasm around the nuclear figure.

FIG. 18. Late metaphase, after splitting of the chromosomes, very clearly defined spindle.

FIG. 19. Anaphase, spindle still clearly defined.

FIG. 20. Telophase, trace of spindle in equatorial region.

FIG. 21. Spore mother-cell, daughter nuclei after first mitosis in resting condition, four chloroplasts.

FIG. 22. Nuclear plate at first mitosis, side view.

FIG. 23. Nuclear plate of second mitosis viewed from pole of spindle, indicating longitudinal splitting of the chromosomes.

FIG. 24. Adjacent sections of same spore-mother-cell during second mitosis; nuclear plate of one spindle with four chromosomes viewed from the end; side view of the other spindle.

FIG. 25. Center of spore-mother-cell showing three nuclei, cytoplasm segregated around the nuclei and chloroplasts, delicate strands connecting the regions.

FIG. 26. First indication of the position of the walls that are to be formed in the spore-mother-cell; the coalescence of connecting strands of cytoplasm to form a delicate film.

FIG. 27. Final separation of the protoplasmic contents of the spore-mother-cell.